WO 2005/010166

- 52 -

CLAIMS

We claim:

15

25

- 1. An isolated protein, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2, wherein the protein has taxoid oxygenase activity.
 - 2. The isolated protein of claim 1, comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 2.
- The isolated protein of claim 1, comprising an amino acid sequence as set forth in SEQ ID NO: 2.
 - 4. The isolated protein of any one of claims 1-3, wherein the taxoid oxygenase activity comprises taxadiene hydroxylation.
 - 5. An isolated nucleic acid molecule encoding the protein of claim 1, 2, 3, or 4.
 - 6. An isolated nucleic acid of claim A5 encoding the protein of claim 1.
- 7. An isolated nucleic acid molecule according to claim 5 comprising a sequence as set forth in SEQ ID NO: 1.
 - 8. A recombinant nucleic acid molecule, comprising a promoter sequence operably linked to a nucleic acid molecule according to claim 5.
 - 9. A cell transformed with a recombinant nucleic acid molecule according to claim 8.
 - 10. The cell of claim 9, wherein the cell is a plant cell, an insect cell, a bacterium, or a yeast cell.
 - 11. The cell of claim 10, wherein the cell is a plant cell from the genus Taxus.
 - 12. The cell of claim 9, wherein the cell is an isolated cell!
- 35 13. A non-human transgenic organism, comprising a recombinant nucleic acid molecule according to claim 8.
 - 14. The organism of claim 13, wherein the organism is a plant.

WO 2005/010166 PCT/US2004/023656

- 53 -

- 15. The organism of claim 14, wherein the plant is from the genus Taxus.
- An isolated nucleic acid molecule that hybridizes under high stringency conditions with a nucleic acid probe comprising at least 600 base pairs of SEQ ID NO: 1, wherein the nucleic
 acid molecule encodes a protein having taxoid oxygenase activity.
 - 17. An isolated nucleic acid molecule according to claim 16 that hybridizes under very high stringency conditions.
- 10 18. The protein encoded by the nucleic acid molecule of claim 17.
 - 19. A recombinant nucleic acid molecule, comprising a promoter sequence operably linked to the nucleic acid molecule of claim 16.
- 15 20. A cell transformed with a recombinant nucleic acid molecule according to claim 19.
 - The cell of claim 20, wherein the cell is a plant cell, an insect cell, a bacterium, or a yeast cell.
 - 22. The cell of claim 21, wherein the cell is a plant cell from the genus Taxus.
 - 23. The cell of claim 21, wherein the cell is an isolated cell.

20

- 25 24. A non-human transgenic organism, comprising a recombinant nucleic acid molecule according to claim 19.
 - 25. The organism of claim 24, wherein the organism is a plant.
- The organism of claim 25, wherein the plant is from the genus Taxus.
 - An isolated nucleic acid molecule, comprising a sequence having at least 80% sequence identity with SEQ ID NO: 1, wherein the nucleic acid molecule encodes a protein having taxoid oxygenase activity.
 - 28. An isolated nucleic acid molecule according to claim 27, comprising the sequence set forth in SEQ ID NO: 1.
 - 29. The protein encoded by the nucleic acid molecule of claim 28.

10

25

- 30. A recombinant nucleic acid molecule, comprising a promoter sequence operably linked to the nucleic acid molecule of claim 27.
- 5 31. A cell transformed with a recombinant nucleic acid molecule according to claim 30.
 - 32. The cell of claim 31, wherein the cell is a plant cell, an insect cell, a bacterium, or a yeast cell.
 - 33. The cell of claim 32, wherein the cell is a plant cell from the genus Taxus.
 - 34. The cell of claim 32, wherein the cell is an isolated cell.
- 15 35. A non-human transgenic organism, comprising a recombinant nucleic acid molecule according to claim 30.
 - 36. The organism of claim 35, wherein the organism is a plant.
- 20 37. The organism of claim 36, wherein the plant is from the genus Taxus.
 - 38. A method of identifying a nucleic acid sequence that encodes a taxoid oxygenase, comprising:
 - (a) hybridizing a probe to a nucleic acid sequence under high stringency conditions, wherein the probe comprises at least 600 contiguous nucleotides of SEQ ID NO: 1; and
 - (b) determining that a protein encoded by the nucleic acid sequence is capable of oxidizing a taxoid substrate, wherein a protein capable of oxidizing a taxoid substrate is identified as a taxoid oxygenase.
- 30 39. The method of claim 38, wherein step (a) is performed under very high stringency conditions.
 - 40. The method of claim 38, wherein oxidizing the taxoid substrate comprises hydroxylating the taxoid substrate.
 - 41. A method of hydroxylating a substrate, comprising:

 contacting a substrate with at least one oxygenase comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 2; and allowing the oxygenase to oxidize the substrate.

WO 2005/010166 PCT/US2004/023656

- 55 -

- 42. The method of claim 41, wherein oxidation of the substrate comprises hydroxylation of the substrate.
- 5 43. The method of claim 41, wherein the substrate is a taxoid.
 - 44. The method of claim 43, wherein the taxoid is paclitaxel or a paclitaxel intermediate.
- 10 45. The method of claim 43, wherein the taxoid is a taxadiene.

30

- 46. The method of claim 45, wherein the taxadiene is taxa-4(5),11(12)-diene or taxa-4(20),11(12)-diene.
- 15 47. The method of claim 43, 44, 45, or 46, wherein the oxygenase hydroxylates position C5 of the taxoid.
 - 48. The method of claim 41, wherein the amino acid sequence is SEQ ID NO: 2.
- 20 49. The method of claim 41, 43, 44, 45, 46, or 48, wherein the oxygenase is expressed in an isolated cell or in a transgenic plant, bacterium, insect, fungus or yeast, and the hydroxylation of the substrate occurs in vivo.
- 50. The method of claim 49, wherein the substrate is an exogenous substrate, which is fed to the isolated cell, transgenic plant, transgenic bacterium, transgenic insect, transgenic fungus or transgenic yeast.
 - 51. A method for increasing paclitaxel yield in a cell, comprising introducing at least one nucleic acid molecule of claim 6, 16, or 27 into a paclitaxel-producing cell, wherein an amount of paclitaxel produced by the cell increases following introduction of the at least one nucleic acid molecule into the cell.
 - 52. The method of claim 51, wherein introducing the at least one nucleic acid molecule into the cell comprises transfecting the cell with the at least one nucleic acid molecule.
 - 53. The method of claim 51, wherein the at least one nucleic acid molecule comprises a sequence as set forth in the protein-coding region of SEQ ID NO: 1.

- 54. The method of claim 51, wherein the paclitaxel produced by the cell increases by at least two fold.
 - 55. The method of claim 51, wherein the cell is a Taxus cell.

5

- 56. The method of claim 51, further comprising introducing into the cell an additional nucleic acid molecule selected from the group consisting of:
- a first nucleic acid sequence encoding a protein having taxadiene synthase (a) activity;
- 10 **(b)** a second nucleic acid sequence encoding a protein having taxadien-5-ol transacylase activity;
 - (c) a third nucleic acid molecule encoding a protein having taxadien-2-ol transacylase activity;
- one or more fourth nucleic acid molecules encoding one or more proteins 15 having taxoid oxygenase activity;
 - a fifth nucleic acid molecule encoding a protein having 10-deacetylbaccatin III-10-O-acetyl transferase activity;
 - a sixth nucleic acid molecule encoding a protein having taxoid 13-phenylpropanoyltransferase activity;

20 (g)

- a seventh nucleic acid molecule encoding a protein having 3'-N-debenzoyltaxol N-benzoyltransferase activity; and
 - combinations of (a), (b), (c), (d), (e), (f), or (g). (h)

57. The method of claim 56, wherein:

25

30

- (a) the first nucleic acid molecule comprises a nucleic acid sequence having at least 90% sequence identity to the protein-coding region of SEQ ID NO: 19 and encodes a protein having taxadiene synthase activity:
- the second nucleic acid molecule comprises a nucleic acid sequence having at least 90% sequence identity to the protein-coding region of SEQ ID NO: 21 and encodes a protein having taxadien-5-ol transacylase activity;
- the third nucleic acid molecule comprises a nucleic acid sequence having 90% sequence identity to the protein-coding region of SEQ ID NO: 23 and encodes a protein having taxadien-2-ol transacylase activity;
- the one or more fourth nucleic acid molecules comprise a nucleic acid (d) sequence having 90% sequence identity to any one of the sequences set forth in SEQ ID NOs: 3, 5, 7, 9, 11, 13, 15, 17, and 40 and encode a protein having taxoid oxygenase activity;
- the fifth nucleic acid molecule comprises a nucleic acid sequence having 90% sequence identity to the protein-coding region of SEQ ID NO: 34 and encodes a protein having 10-deacetylbaccatin III-10-O-acetyl transferase activity;

10

20

- (f) the sixth nucleic acid molecule comprises a nucleic acid sequence having 90% sequence identity to the protein-coding region of SEQ ID NO: 36 and encodes a protein having taxoid 13-phenylpropanoyltransferase activity; and
- (g) the seventh nucleic acid molecule comprises a nucleic acid sequence

 having 90% sequence identity to the protein-coding region of SEQ ID NO: 38 and encodes a protein having 3'-N-debenzoyltaxol N-benzoyltransferase activity.
 - 58. The method of claim 56, wherein the one or more fourth nucleic acid molecules encode one or more proteins having taxoid 7β -hydroxylase activity, taxoid 14β -hydroxylase activity, taxoid 10β -hydroxylase activity or taxoid 13α -hydroxylase activity.
 - 59. The method of claim 56, wherein introducing the additional nucleic acid molecule into the cell comprises transfecting the cell with the additional nucleic acid molecule.
- 15 60. The method of claim 56, wherein the additional nucleic acid molecule is selected from the group consisting of:
 - (a) a first nucleic acid molecule comprising a nucleic acid sequence having at least 90% sequence identity to the protein-coding region of SEQ ID NO: 19, wherein the first nucleic acid sequence encodes a protein having taxadiene synthase activity;
 - (b) a second nucleic acid molecule comprising a nucleic acid sequence having at least 90% sequence identity to the protein-coding region of SEQ ID NO: 21, wherein the second nucleic acid sequence encodes a protein having taxadien-5-ol transacylase activity;
 - (c) a third nucleic acid molecule comprising a nucleic acid sequence having 90% sequence identity to the protein-coding region of SEQ ID NO: 23, wherein the third nucleic acid sequence encodes a protein having taxadien-2-ol transacylase activity; and
 - (d) combinations of (a), (b), or (c).
 - 61. The method of claim 60, wherein the additional nucleic acid molecule is selected from the group consisting of:
- a first nucleic acid molecule comprising the protein-coding region of SEQ ID NO: 19;
 - (b) a second nucleic acid molecule comprising the protein-coding region of SEQ ID NO: 21;
- (c) a third nucleic acid molecule comprising the protein-coding region of SEQ 35 ID NO: 23; and
 - (d) combinations of (a), (b), or (c).
 - 62. The method of claim 51, wherein the cell is a cell line.

- 63. An antibody or antibody fragment that binds the protein of claim 1, 18, or 29.
- 64. The antibody of claim 63, wherein the antibody is a monoclonal antibody.
- 5 65. The antibody fragment of claim 63, wherein the antibody fragment is a Fab, F(ab)2, or Fv fragment, or a combination thereof.